Clinically relevant humanized mouse models of metastatic prostate cancer facilitate therapeutic evaluation

Raymond J Kostlan[1], John T Phoenix[1], Audris Budreika[1], Marina G Ferrari[1], Neetika Khurana[1], Jae E Choi[2,3,4], Kristin Juckette[2,3,4], Somnath Mahapatra[2,3,4], Brooke L McCollum[2,3,4], Russell Moskal[1], Rahul Mannan[2,3,4], Yuanyuan Qiao[2,3,4], Donald J Vander Griend[5], Arul M Chinnaiyan[2,3,4], and Steven Kregel
Raymond Kostlan[1], Marina Ferrari[1], John T. Phoenix[1], Audris Budreika[1], Neetika Khurana[1], Jae Eun Cho[2,3,4], Yuanyuan Qiao[2,3,4], Arul M. Chinnaiyan[2,3,4], and Steven Kregel[1].

1 Department of Cancer Biology, Loyola University Chicago, Maywood, IL 60153; 2 Michigan Center for Translational Pathology, University of Michigan; 3 Department of Pathology, University of Michigan; 4 Rogel Cancer Center, University of Michigan Medical School, Ann Arbor, MI 48109, USA; 5 Department of Pathology, University of Illinois at Chicago, Chicago, IL 60612

Background: There exists a tremendous need for establishing prostate cancer (PCa) murine models capable of recapitulating the progress of human PCa. By itself, the murine prostate does not form sporadic tumors and is anatomically and developmentally different from the human prostate. Engineered mouse models, however, lack the heterogeneity of human disease, are often driven in a contrived manner, and rarely (if ever) establish metastatic growth. Human xenografts represent an alternative, but they rely on tumor growth in an immunocompromised host, thus preventing the study of tumor-immune interactions and immunotherapies. Accordingly, we generated PCa xenograft models in a murine system with an intact human immune system (huNOG mice) to test the hypothesis that humanizing tumor-immune interactions would improve modeling of metastatic PCa as well as our ability to model the impact of hormonal and immunotherapies.

Methods: Male huNOG and huNOG-EXL mice were produced at Taconic Biosciences by engrafting juvenile NOG mice with human CD34+ hematopoietic stem cells. These mice stably maintained multiple human cell lineages, including functional human T-cells. We utilized two human PCa xenograft cell line models transduced with luciferase to assay organ-specific metastatic growth. First, castrated and intact control mice were injected subcutaneously with 22Rv1 cells. When tumors reached >100 mm^3, half of the castrated mice were treated with enzalutamide (enza), and tumor growth was then monitored to endpoint. Additionally, VCaP tumor–bearing mice were castrated when the tumors reached ~200 mm^3, and once the tumor grew back, they were randomized and treated with enza and/or the anti-PD-1 antibody, pembrolizumab (pembro) as well as with vehicle controls. At sacrifice, organs were *ex vivo* analyzed for metastatic growth, tumor infiltrating lymphocytes, and splenic immune reconstitution.

Results: In 22Rv1 tumor-bearing mice, subcutaneous tumor size was not significantly altered across conditions. However, the extent and growth at the secondary sites differed markedly in castrate huNOG vs conventional NOG mice treated with enza. VCaP xenograft tumors showed marked decreases in growth with enza and pembro treatments in huNOG mice, while no effect was observed with either of these treatments in NOG mice. Furthermore, immune responses to enza treatment in huNOG and NOG mice were distinct, as enza treatment only increased intra-tumor CD3+ T-cells as well as CD3+ T-cell activation in huNOG mice. Myeloid support in huNOG-EXL promoted Myeloid-Derived-Suppressor-Cell-like (MDSC) differentiation, blunting anti-tumor responses.

Conclusions: These results illustrate, to the best of our knowledge, the first model of human PCa that metastasizes to clinically relevant locations, has an intact human immune system, and responds to standard-of-care hormonal therapies in a manner similar to responses observed under clinical settings.

Funding Acknowledgements: 2019 Robert Citrone, George Walker, & Mark Weinberger – Prostate Cancer Foundation VAlor Young Investigator Award. **Conflicts of Interest Disclosure Statement:** Taconic Biosciences provided resources.